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Control of drinking water by linking biosensors with physicochemical methods

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Abstract

An alarm system is developed for the direct detection of toxic substances in drinking water. The vitality of living organisms or cells is monitored continuously by electrochemical and infrared spectroscopic methods. Mammalian cells or bacteria are acting as highly sensitive biosensors and are monitored simultaneously by infrared and electrochemical techniques. It enables the non-specific detection of toxins with short response times for implementation in water supplies.

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Keywords: biosensor, infrared spectroscopy, water quality monitoring, electrochemical detection, diamond

1. Introduction

Water is fundamental to our quality of life, to economic growth, and to the environment. Water pipes, however, permanently take the risk of being contaminated. For example, repairs, industrial accidents or pesticides from agriculture may cause contamination of groundwater and finally, drinking water supply systems. Various physical and chemical approaches have been developed to evaluate water quality [1] including the detection of indicated properties of water samples such as color, turbidity, odor and taste as well as pH and hardness of water.

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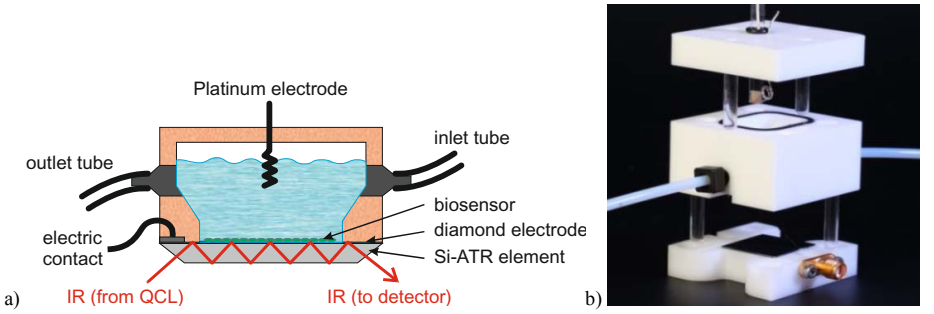

Fig. 1: (a) Scheme of the flow cell, (b) Exploded view of the measurement cell (without laser module and detector).

Most of these methods and sensors are only designed to identify individual targets or species and cannot serve as early warning indicators for hazardous contamination of water. The goal of this work is the demonstration of a novel system for real time monitoring water quality, but without specific regard to precision, accuracy or identification of the contaminant. The aim is to generate an alarm signal due to the toxic effect on biosensors. It makes use of the fact that the biological activity of living cells is species-specifically impaired by cytotoxic substances. This either affects the interactions at interfaces (neighboring cells, material surfaces), or results in a measurable change to cellular components. As a consequence, alarm can be triggered for any hazardous impact on the metabolism of the cells, without the necessity of exact knowledge about its origin. Such biosensors consisting of living cells can be selected and adapted to meet individual requirements such as the prompt and comprehensive monitoring of the quality of water. The major advantages of biosensors are (i) the possibilities for in situ monitoring, (ii) a fast response, and (iii) the reaction on the toxic effect of chemical substances or biological toxins, thus, avoiding false alarm in case of harmless substances. Recently, genetically modified fluorescent organisms are used as whole-cell biosensors and reduction in fluorescence as a response to the presence of a toxic substance is tracked quantitatively [2]. In this work, alternative methods to monitor the metabolism of genetically non-modified cells are being developed. The cell response on cytotoxic substances is monitored by means of impedance measurements and supplemented by infrared (IR) spectroscopy, which detects chemical effects on proteins, amino acids and nucleic acids. Both measuring methods were successfully integrated into a measuring cell suitable for online monitoring (Fig. 1).

2. Diamond electrode

The biosensors are immobilized on a boron-doped diamond film, which is one of most stable electrode materials with several attractive features such as wide electrochemical potential window, low background current and high chemical stability in almost all media [3]. The most important feature for biosensors is its biocompatibility. In addition, an electrochemical method can be employed to avoid biofouling on the surface [4]. In the flow cell, the conductive diamond film acts as electrochemical electrode and simultaneously couples IR light to the biosensor. To enhance the efficiency of the IR spectroscopy, the diamond film is grown on top of an attenuated total reflectance (ATR) element. To adapt the spectral range given below, a silicon based ATR element was employed and covered with 200 nm boron-doped diamond, which has negligible impact on the IR transmission at the selected wavelengths. The conductive nanocrystalline diamond films were grown by microwave-enhanced chemical vapor deposition at 750 °C with 2 % methane and 5000 ppm trimethylboron as doping gas [5]. Before placing them into the system, the diamond samples were cleaned in H_2SO_4:HNO_3 (3:1, 250 °C for 2 h) and thoroughly rinsed in water.

3. Biosensors

To determine the toxic effects of substances, mammalian cells and microorganisms were used as biological sensors integrated in the flow cell. Two harmless types of bacteria have been selected: *Caulobacter vibrioides* (formerly *Caulobacter crescentus*) which is a ubiquitous species usually found in fresh water lakes and streams; the second one was a non-pathogenic strain of *Escherichia coli*.

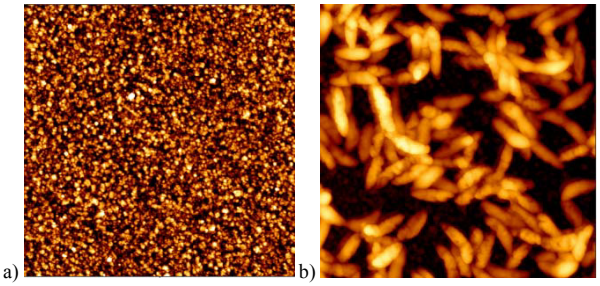

Fig. 2: AFM image of a nanocrystalline diamond surface in air before (a) and after (b) growing *Caulobacter vibrioides* (10×10 µm²).

Bacteria were grown directly on diamond in a bioreactor with a flow system. The concentration of bacteria in the bioreactor was 4.4×10^6 cells per ml. The medium was prepared separately with pH 7.15. The flow cells (Fig. 1) were rinsed with ethanol and fermented at a working temperature of 20 °C. Tapping mode atomic force microscopy (AFM) as well as scanning electron microscope imaging was employed to confirm the growth of bacteria on the diamond surface. The bacteria were fixed with glutaraldehyde (2 %) buffer solution. The comparison of AFM images before (a) and after (b) the growth of the bacteria demonstrates a homogeneous distribution on a smooth diamond surface. AFM images of bacteria taken in tap water without fixation are similar with respect to density, size and shape of the bacteria.

4. IR spectroscopy

IR spectroscopy is a powerful tool for the analysis and identification of biological systems. Most relevant organic molecules as well as microbial cells have prominent peaks in this spectral range. Its application in water is limited due to strong absorption. High spectral power density of IR external cavity quantum cascade lasers (EC-QCL) and the application of ATR, however, enable its use in water. Fig. 3a) shows the obvious alteration in the IR spectra of bacterial cells in the presence of a toxin. Glutaraldehyde connects primary and secondary amines irreversibly and therefore affects the amide I and II bands in the area of W1'. This is clearly visible in the min-max-normalized spectra (A) as well as, and even with a better resolution, in the second derivation of the absorption bands (B). The amide I band has moved six wave numbers from 1636 cm^{-1} to 1642 cm^{-1}. This indicates a change in secondary protein structures. Similarly, the reaction of mammalian HEK cells on the exposure to the substance acrylamide has a clear impact on the IR spectra (Fig. 3b). Acrylamide reacts with amino and nucleic acids, which can be seen in the change of the IR spectrum at the relevant wavelengths 1500-1700 cm^{-1} and 1050-1250 cm^{-1}, respectively.

Consequently, most prominent detectable changes are found on the amide bands in the spectral region between 1450 and 1720 cm^{-1}. For this region, a tunable laser source is realized based on a homemade EC-QCL [6] (Fig. 4). The sharp dips in the spectra are due to atmospheric absorption and are suppressed in the integrated sensor system.

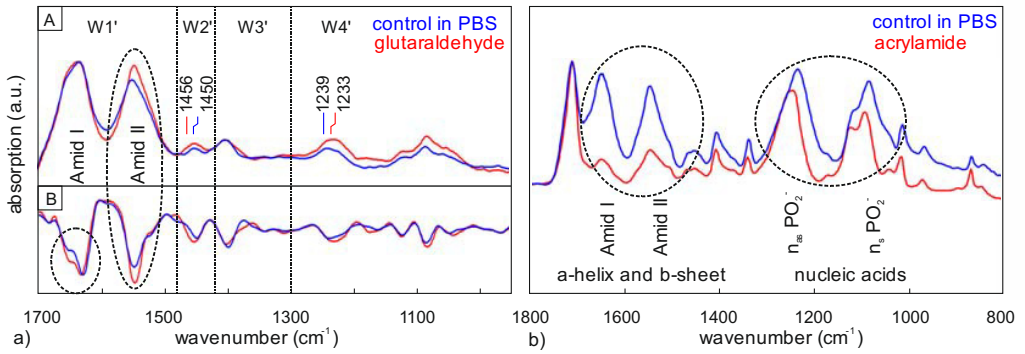

Fig. 3: a) IR spectra of *C. vibrioides* cells exposed to glutaraldehyde (red) compared to unaffected cells (control in PBS, blue) and the normalized second derivation of the spectra; b) Fig. 4: IR spectra of HEK cells exposed to acrylamide compared to unaffected cells (Control in PBS).

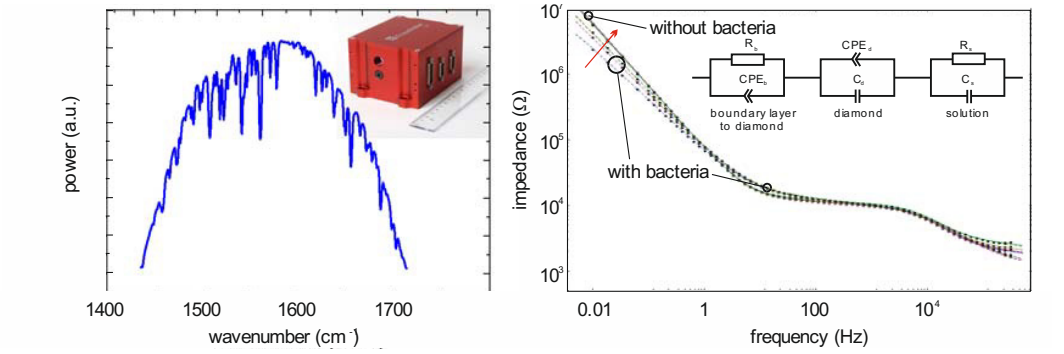

Fig. 4: a) Tuning range of the implemented QCL (Insert: complete homemade laser module); b) Impedance spectra for diamond and diamond overgrown with *E. coli* in synthetic tap water. Red arrow indicates increasing of the ethanol concentration in a system with bacteria, which results in a clear rise of the impedance in the low frequency region, while the system without bacteria is not affected. Insert: Electric equivalent circuit, where the components are assigned to its possible origin.

5. Electrochemical measurements

Electrochemical measurements were performed in synthetic tap water with a Pt-coil as counter and reference electrode. The working electrode was either a bare boron-doped diamond or the bacteria/diamond electrode. Voltammograms in water and 0.1 M KCl buffer were compared in the potential range of -0.2 to +0.7 V (vs. Pt). No influence of the solution on the measurements could be identified.

Impedance spectroscopy was applied to investigate surface properties of the bacteria/diamond interface (Fig. 4b). To avoid changing the bacteria/diamond interface and the morphology of the bacteria under electric fields, the open circuit potential was applied. The different sections in the spectra are modelled by an equivalent circuit using resistors R, capacitances C and constant phase elements CPE (Fig. 4b, inset). The growth of bacteria on the diamond causes a change in the parameters of the constant phase element that describes the boundary layer to the diamond, resulting in a decrease of the absolute value of the impedance in the low frequency region. Adding cytotoxins restores the initial conditions of the system without bacteria. The mechanism generating the impedance shift upon bacteria growth and cytotoxin dosing could not yet be clearly identified.

6. Summary

In summary, an alarm system is developed for the direct detection of toxic substances in drinking water. It is based on the monitoring of the vitality of living organisms or cells by continuous electrochemical and infrared spectroscopic methods. As biosensors, Mammalian cells or bacteria can be employed and boron-doped nanocrystalline diamond is a suitable substrate for those living cells. Both, impedance and IR spectroscopy clearly show the presence of the cells on the diamond surface. The impact of cytotoxins is detected by changes of the typical IR signatures; impedance spectroscopy shows alterations in low and high frequency range, whose origin could not yet be clarified. Together with a more detailed study of different toxic substances, this will be the subject of further investigations.

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